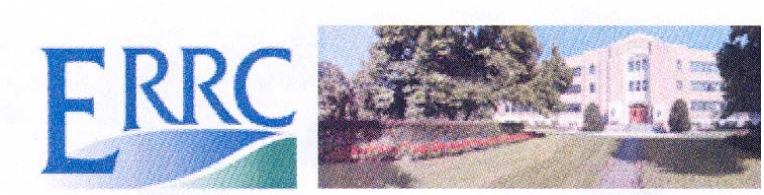

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# Surface Pasteurization of Whole Fresh Cantaloupes Inoculated with *Salmonella* Poona or *Escherichia coli*†

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## ABSTRACT

Numerous outbreaks of salmonellosis by *Salmonella* Poona have been associated with the consumption of cantaloupe. Commercial washing processes for cantaloupe are limited in their ability to inactivate or remove this human pathogen. Our objective was to develop a commercial-scale surface pasteurization process to enhance the microbiological safety of cantaloupe. Populations of indigenous bacteria recovered from cantaloupes that were surface pasteurized at 96, 86, or 76°C for 2 to 3 min were significantly ($P < 0.05$) lower than those of the controls. Whole cantaloupes, surface inoculated with *Salmonella* Poona RM 2350 or *Escherichia coli* ATCC 25922 to a final cell concentration of ca. 5 log CFU/cm$^2$ were stored at 4°C or room temperature (RT = 19 ± 1°C) for up to 72 h before processing. Treatments at 76°C for 2 to 3 min at 24 h postinoculation resulted in a reduction in excess of 5 log CFU/cm$^2$ of *Salmonella* Poona and *E. coli* populations. Cantaloupes that were surface pasteurized and stored at 4°C for 21 days retained their firmness qualities and had no visible mold growth compared with the controls, which became soft and moldy. These results indicate that surface pasteurization will enhance the microbiological safety of cantaloupes and will extend the shelf life of this commodity as well. Storage of untreated inoculated cantaloupes at RT for 24 to 72 h postinoculation caused a significant ($P < 0.05$) increase in *Salmonella* Poona and *E. coli* populations compared with storage at 4°C. This indicates that cantaloupes should be refrigerated as soon as possible following harvest to suppress the growth of any possible contaminant on the rind.

*Salmonella* is the most frequently reported cause of foodborne illnesses *(6)*, and it results in up to 2,000 deaths annually in the United States *(13)*. Although foodborne salmonellosis is often associated with foods of animal origin, *Salmonella* has been isolated from a wide variety of fruits and vegetables *(3)*. Also, Wells and Butterfield *(29)* isolated *Salmonella* from 18 to 20% of produce samples with decay collected from the marketplace and from 9 to 10% of healthy produce samples, and they reported that growth of spoilage microorganisms in produce tissues enhanced the growth of *Salmonella*. Cantaloupe has been identified as a vehicle for *Salmonella* infection implicated in foodborne salmonellosis in the United States and Canada and has resulted in more than 800 cases between 1990 and 2000 *(23)*. A recent survey conducted by the U.S. Food and Drug Administration (FDA) on domestic fresh produce reported an incidence of 2.4% positive for *Salmonella* and 0.6% positive for *Shigella* in 115 samples of cantaloupe *(26)*. In a FDA survey of imported fresh produce *(24)*, 5.3 and 2.0% of 151 cantaloupe samples were positive for *Salmonella* and *Shigella*, respectively. In 2001 and 2002, two deaths in the United States and numerous cases of salmonellosis in the United States and Canada were associated with the consumption of Mexican cantaloupes contaminated with *Salmonella* Poona *(5)*. This led to the issuance of an import alert on all Mexican cantaloupes by the FDA *(25)* and the Canadian Food Inspection Agency *(5)* until importers could certify that Mexican cantaloupes were produced under proper sanitary conditions.

Cantaloupe, normally grown on the ground, can become contaminated with a human pathogen such as *Salmonella* anytime during production. The presence of pathogens on the rind of the cantaloupe has implications for safety applicable to fresh-cut fruit markets. This led the FDA *(25)* to issue guidelines designed to help keep fruit fresh and safe for processors and consumers that outline safe handling processes for fresh produce, including cantaloupe. Included in the guidelines is a thorough washing with cold tap water of cantaloupe rind before cutting. Washing studies of cantaloupe that used different produce sanitizing agents were only partially effective in reducing microbial populations *(18, 19, 21, 22)*. Survival of *Salmonella* and other bacteria during washing treatments could be attributed to their attachment to inaccessible sites on the rind, such as the netting *(14, 27)*; infiltration within the stem scar, as with apples *(1, 2)*; and incorporation into biofilms, as with apples *(2)* and leaf surfaces *(4, 9)*. Thus, inadequate decontamination of cantaloupe can result in survival of the pathogen and transfer from the rind to the flesh during fresh-cut processing. Furthermore, human pathogens have been shown to survive and grow on fresh-cut cantaloupe and watermelon during storage *(7, 11)*. Thus, the safety of fresh-cut cantaloupe in supermarkets and salad bars is a concern. Research is needed to develop new technologies

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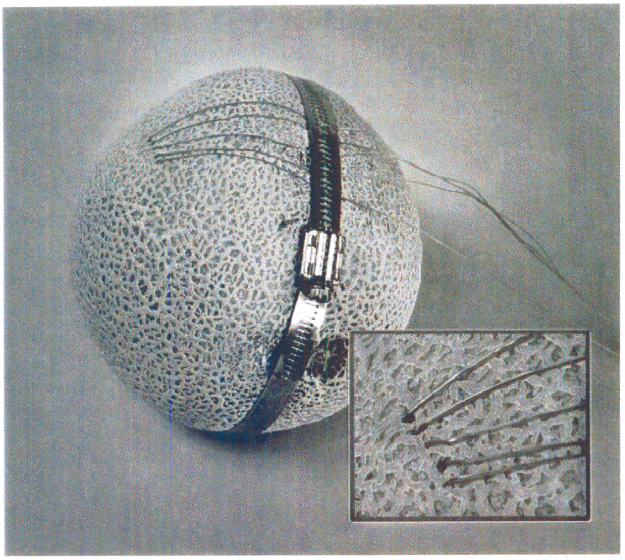

FIGURE 1. *Five thermocouples embedded in a cantaloupe at varying depths (surface to 12.7 mm). The clamp was used to prevent the thermocouple from being pulled out of the cantaloupe during processing. Inset picture is an enlargement of embedded thermocouple locations.*

capable of inactivating microorganisms in their protective attachment states on the surface of cantaloupe.

Although thermal treatment or pasteurization is the most effective method for destroying microorganisms, it is not usually used in the fresh-cut produce industry *(16)*. Ukuku et al. *(21)* indicated that immersion of cantaloupe in hot water or 5% hydrogen peroxide solution at 70°C for 60 s resulted in up to a 3.8-log reduction in *Salmonella* populations. Although thermal treatment of a wide variety of fruits and vegetables with hot water *(2, 10, 12, 15–17, 21)* or steam *(13, 20)* resulted in significant improvement in microbiological qualities of the produce, it significantly reduced seed germination *(12)* and resulted in thermal injury to apples *(17)*. Most of these experiments were carried out under laboratory conditions, and treatment parameters were not optimized. The objectives of this study were to develop and optimize a surface pasteurization (hot water) process with commercial-scale processing equipment and to determine treatment effects on melon quality and shelf life under conditions simulating commercial production. Also, we sought to compare this commercial-scale surface pasteurization process to that of a laboratory-scale process used for studies with human pathogens.

## MATERIALS AND METHODS

**Determination of thermal penetration profile in cantaloupe during the surface pasteurization process.** Five 30-gauge (1.8 m long) type "T" thermocouples (Omega Engineering, Stamford, Conn.) were embedded into a cantaloupe (Fig. 1) at various depths (surface level to 12.7 mm) and connected to the data acquisition system, which comprised five Accutech smart transmitters (model AI-1000 R2, Accutech, Hudson, Mass.), one National Instruments (NI, Austin, Tex.) screw terminal board, one NI DAQ card model PCI-6024E, NI Lab View software version 6.0, and a Pentium III computer. Cantaloupes, one at a time, were washed with agitation in tap water (450 liters) at 96, 86, or 76°C for 8 min in a commercial-scale pasteurization tunnel PT-1030 (Gel-Ash Stainless Steel Products Ltd., Holon, Israel) and was rapidly cooled in an ice water bath for 5 min.

**Bacterial strains, maintenance, growth conditions, and inoculum development.** *Salmonella* Poona RM 2350 (California Department of Health Services 00A3563), a clinical isolate associated with cantaloupe outbreak, was obtained from Dr. William Fett (U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center [USDA-ARS-ERRC], Wyndmoor, Pa.). *Escherichia coli* ATCC 25922 was obtained from American Type Culture Collection (Manassas, Va.). Stock cultures were stored in tryptic soy broth (TSB; BBL/Difco, Sparks, Md.) containing 20% glycerol at −80°C. Working stocks were maintained on tryptic soy agar (TSA; BBL/Difco) slants containing 0.6% yeast extract stored at 4°C for 2 to 4 weeks. A loopful of culture from a TSA slant was transferred into 10 ml of TSB and allowed to grow for approximately 8 h at 37°C. This culture was then used to inoculate 2 liters of the same medium at a 0.01% (vol/vol) level. The culture was allowed to grow to stationary phase at 37°C, spun down at 6,740 × *g* for 20 min, washed once with 400 ml sterile deionized water, and suspended in 4 liters of sterile deionized water to give a final cell concentration of ca. 9 log CFU/ml. The inoculum was stable in deionized water for at least 24 h, as seen by the recovery of similar microbial cell densities at time 0, 4, and 24 h.

**Inoculation of cantaloupe.** Cantaloupes (*Cucumis melo* L.; 12.7 by 13.4 cm to 16.0 by 14.4 cm, weighing between 1,134 g and 1,764 g) free of decay and punctures were obtained from local suppliers and grocery stores and stored at 4°C for no more than 3 days before use. These cantaloupes were inoculated by a dip method whereby cantaloupes were submerged (one cantaloupe at a time) in 4 liters of inoculum for 5 min then drained and allowed to air dry on absorbent paper for 1 h on each side in a biosafety cabinet. Cantaloupes were then stored in plastic tubs lined with absorbent paper at room temperature (RT = 19 ± 1°C) or 4°C for up to 72 h.

**Washing and sampling protocols.** Unless otherwise indicated, all washing protocols were carried out in tap water with commercial-scale equipment or a laboratory-scale water bath. The commercial-scale washing process was carried out in the PT-103 pasteurization tunnel containing 450 liters of water. The laboratory-scale washing process was done in an 8-liter covered glass jar containing 4 liters of water placed in an Innova 3100 water bath shaker (New Brunswick Scientific Co. Inc., Edison, N.J.) with shaking at 120 rpm. Triplicate samples, each consisting of a single cantaloupe, were totally immersed in wash water at a specific temperature for a specific preset residence time, immediately sealed in plastic bags, and submerged in an ice water bath for 5 min. Most of the air in the plastic bag was removed before sealing to ensure proper heat transfer during the cooling process. Whole rinds of cantaloupes were then analyzed for residual surface microbial populations. The whole rind (flesh-free) was removed with a sterile Muro Peel-All Fruit Peeler CP-44 (Muro Corporation, Tokyo, Japan), placed in a sterile 1-liter glass blender jar (Waring Products, Torrington, Conn.), combined with four equal volumes (wt/vol) of 0.1% peptone water (PW; BBL/Difco), and blended at medium speed for 1 min with a commercial blender (Waring blender model 51BL31). The resulting blend was filtered through a filter bag (Spiral Biotech, Bethesda, Md.), and duplicate 10-ml volumes were transferred to sterile tubes. Filtrates were diluted in PW as necessary and surface plated on the appropriate growth medium.

**Enumeration of bacteria recovered from cantaloupe.** TSA was used for enumeration of total aerobic microorganisms and as a recovery medium for injured cells of *Salmonella* Poona and *E. coli* on cantaloupe. Enumeration of uninjured *E. coli* and *Salmonella* Poona populations was done with the use of selective media MacConkey agar (MAC; BBL/Difco) and xylose lysine tergitol-4 agar (XLT-4; BBL/Difco), respectively. Recovery medium (TSA) plates were incubated at 37°C for 2 h to allow recovery of injured cells and then overlaid with the appropriate selective medium. All plates were incubated for 24 h at 37°C, and resultant colonies were counted manually (log CFU per $cm^2$ of rind).

**Surface area calculations.** Before inoculation, the polar diameter ($c$, length of the stem to blossom end axis) and the equatorial diameter ($a$, width at its equator) of each cantaloupe were measured with a 50-cm slide caliper (Mantax, Haglöf Sweden AB, Ländsele, Sweden). The surface area of cantaloupe was calculated by the appropriate equation described by Weisstein *(28)* for a prolate spheroid ($c > a$), oblate spheroid ($c < a$), or sphere ($c = a$).

**Scanning electron microscopy.** Cantaloupes were spot inoculated with 10 μl inoculum per spot (20 different spots per cantaloupe) and allowed to air dry for 2 h on an absorbent paper in a biosafety cabinet. Cantaloupes were then stored in plastic tubs lined with absorbent paper at RT for up to 72 h. Noninoculated and inoculated rind plugs where removed with a sterile cork borer (2 cm diameter), and the flesh attached to each rind plug was removed with a sterile knife. Rind plugs were fixed by a vapor glutaraldehyde method instead of the immersion method to avoid washing off dried layers of inoculum on the rind surface. Rind plugs were incubated in plastic petri dishes lined with filter papers saturated with 25% glutaraldehyde and incubated at RT overnight. Samples for scanning electron microscopy (SEM) imaging were further processed as previously described *(26)*.

**Statistical analysis.** Analysis of variance with individual contrasts and Bonferroni $T$ tests were used to determine significant differences ($P < 0.05$) among population means in response to treatments. Unless otherwise indicated, the level of significance used was $P = 0.05$. All statistical analyses and calculations of means and standard deviations were performed by SAS/STAT software (SAS Institute Inc., Cary, N.C.).

## RESULTS AND DISCUSSION

**Temperature penetration profiles.** Thermal penetration curves were determined to evaluate the internal temperature of cantaloupe following surface pasteurization treatment. This information would be useful in determining the ideal temperature–time combination for surface pasteurization of cantaloupe while maintaining fresh quality of the flesh. Cantaloupes were surface pasteurized at 96, 86, and 76°C for up to 8 min and were immediately cooled in an ice water bath for 5 min. Before surface pasteurization treatments, cantaloupe was stored overnight at 4°C or RT. These storage conditions were chosen to simulate packing house or field packing, handling, transport, and storage practices. Figures 2 and 3 show heat penetration curves of cantaloupe that had been stored at 4°C and RT, respectively, before washing at 76, 86, or 96°C. The pasteurization tunnel was equipped with two curtains on each end to prevent heat loss during operation. The initial 60 to 80 s was the time it took the cantaloupe on the conveyor to cross the curtains before entering the water. Once the cantaloupe was

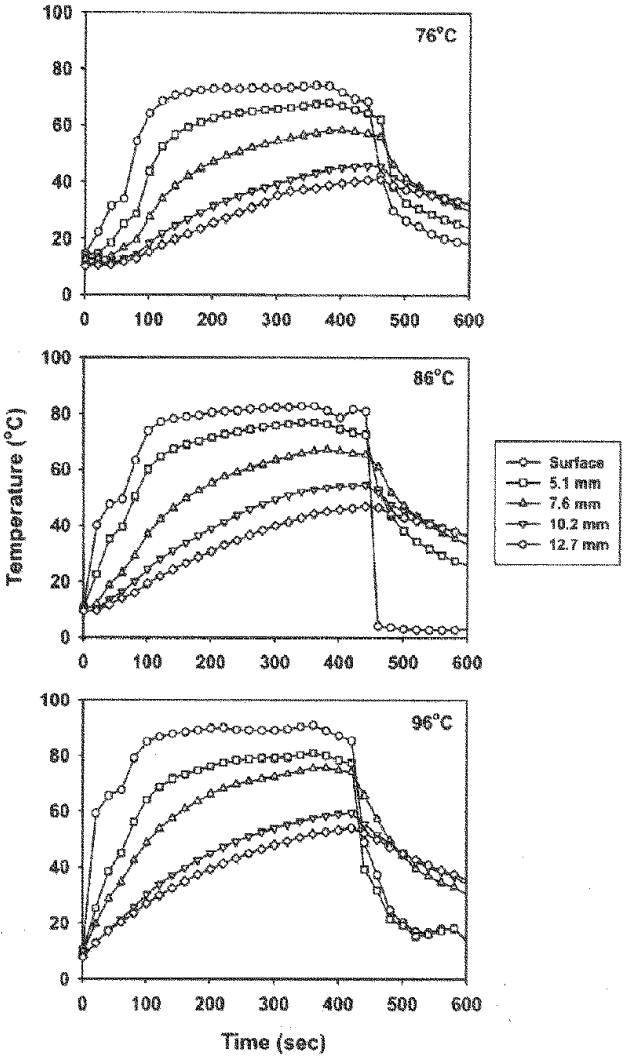


FIGURE 2. *Heat penetration curves of cantaloupe equilibrated to 4°C before submersion in hot water at different temperatures.*

submerged in water, cantaloupe surface and subsurface (5.1 mm) temperatures, but not flesh temperature, increased rapidly. This indicated that the rind insulated the flesh from extreme temperatures. The peel of an orange was reported to provide such insulation for its flesh *(16)*. The maximum surface temperature of cantaloupe was always 2 to 4°C less than the water temperature, irrespective of the cantaloupe initial temperature (Figs. 2 and 3), presumably because the thermocouple was immediately under the rind surface (Fig. 1). Cantaloupe that was stored at 4°C (Fig. 2) before washing had internal temperatures (7.6 to 10.2 mm, respectively) of 10 to 14°C less than cantaloupe stored at RT (Fig. 3). Thus, less heat damage of the flesh would be expected in cantaloupe that was refrigerated before the surface pasteurization treatment. Heat damage was seen in the form of darkening of the flesh just under the rind of cantaloupe that was treated at 96°C for 4 min compared with treatment of 1 to 3 min (data not shown). Cantaloupe that was surface pasteurized and stored at 4°C for 21 days retained its firmness qualities, whereas nontreated cantaloupe became soft. Also, visible spoilage because of mold growth on the surface was seen only on untreated cantaloupes. Thus, this

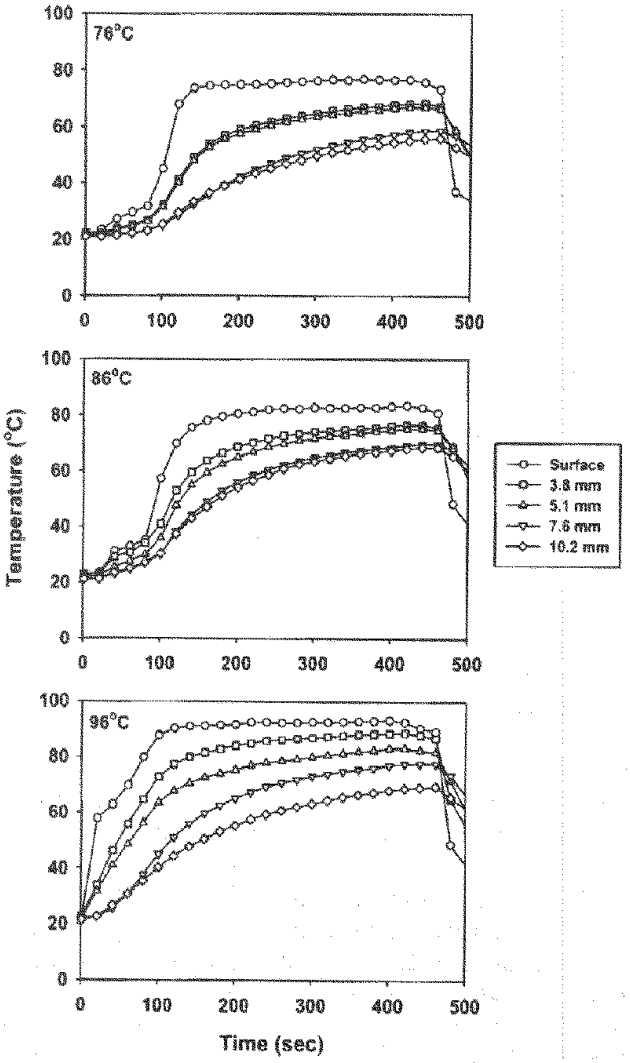


FIGURE 3. *Heat penetration curves of cantaloupes equilibrated to RT before submersion in hot water at different temperatures.*

surface pasteurization process was able to maintain the firmness quality and increase the shelf life of cantaloupe. This increase in shelf life following hot water treatment was reported previously *(15)*.

**Commercial-scale surface pasteurization of cantaloupe.** Residual total aerobic microflora on cantaloupes following surface pasteurization with commercial-scale equipment at different temperatures is shown in Table 1. Overall, surface pasteurization resulted in up to a 2.3-log reduction in indigenous bacteria. Cell densities recovered from control samples were significantly higher than those of surface-pasteurized samples at all temperatures. There was no significant difference in recovered cell densities from all surface-pasteurized samples. The inability of the surface pasteurization process to sterilize the surface of cantaloupe is potentially desirable because the remaining indigenous bacteria could serve as a defense against postprocess cross-contamination with human pathogenic microorganisms.

Previous surrogate screening work from this laboratory *(8)* indicated that *E. coli* ATCC 25922 and *Salmonella* Poona RM 2350 (human pathogen) shared similar growth characteristics on various media, attachment to apples, resistance to hydrogen peroxide washes, and resistance to heat. Therefore, the response of *E. coli* ATCC 25922 to surface pasteurization was compared with that of *Salmonella* Poona RM 2350 under commercial-scale conditions. Cantaloupes were inoculated with *E. coli* and allowed to dry for 24 h at RT or 4°C and surface pasteurized at different temperatures for 2 to 3 min (Table 2). There was no significant change between cell densities of *E. coli* (MAC) and total aerobic plate count (TSA) recovered from control samples and cantaloupes washed at RT for 3 min. This result indicated that the washing treatment with water at RT was not able to dislodge microorganisms attached to the surface of cantaloupes, possibly because of the structure of the rind netting (Fig. 4), which allows microorganisms to attach to inaccessible sites on the cantaloupe and thus avoid contact with the washing water. All surface pasteurization treatments resulted in significant reductions in *E. coli* (MAC) and total aerobic (TSA) cell densities recovered from whole rind of cantaloupes (Table 2). Although surface pasteurization at 76°C for 2 and 3 min did not result in total inactivation of *E.* coli cells on the cantaloupe surface, five of the six treated cantaloupes showed no growth on MAC following the treatment. Also, the magnitude of population reductions following surface pasteurization was significantly larger with MAC (selective) medium compared with TSA (nonselective) medium. Previously, we showed that surface pasteurization did not result in complete inactivation of indigenous microflora on cantaloupe (Table 1). Therefore, microbial cell densities recovered on TSA medium (Table 2) would include the indigenous microorganisms and injured *E. coli* cells. This led us to test for injured cells with recovery medium following surface pasteurization treatment. Recovery of possibly injured *E. coli* cells on nonselective medium following surface pasteurization at 76°C for 2 and 3 min is shown in Table 3. There was a significant reduction in cell populations following surface pasteurization compared with the control. Treatments for 3 min were significantly more effective in reducing *E. coli* populations on cantaloupes compared with 2-min treatments. There was no significant difference between enumeration of *E. coli* populations on selective (MAC) and on recovery (TSA with MAC overlay) media. This indicated that virtually all cells were inactivated rather than injured.

TABLE 1. *Residual indigenous microbial populations on cantaloupes following surface pasteurization treatments*[a]

| Treatment[b] | Residual microbial population (log CFU/cm$^2$) |
|---|---|
| Control | 6.18 ± 0.09 |
| 96°C for 2 min | 3.88 ± 0.04 |
| 86°C for 2 min | 4.24 ± 0.23 |
| 76°C for 2 min | 3.88 ± 0.37 |
| 76°C for 3 min | 4.00 ± 0.26 |

[a] Indigenous microbial populations were determined as total aerobic count on TSA agar medium. Data are reported as the means ± standard deviations for three cantaloupes.

[b] Cantaloupes were stored at 4°C prior to surface pasteurization treatment at the indicated temperature and time.

TABLE 2. *Residual populations of* E. coli[a] *and total aerobic counts*[b] *on cantaloupes (inoculated with* E. coli *ATCC 25922) following surface pasteurization treatments*[c]

| Treatment[d] | Residual population (CFU/cm$^2$) | | | |
|---|---|---|---|---|
| | 4°C | | Room temperature | |
| | MAC | TSA | MAC | TSA |
| 2-h control | 4.18 ± 0.37 (4) | 5.57 ± 0.61 (3) | 4.43 ± 0.21 (2) | 5.81 ± 0.21 (2) |
| 24-h control | 3.96 ± 0.59 (4) | 5.99 ± 0.39 (4) | 5.33 ± 0.39 (2) | 6.40 ± 0.36 (2) |
| 96°C for 2 min | NG (2) | 3.19 ± 0.13 (1) | NG (1) | 3.59 ± 0.49 (1) |
| 86°C for 2 min | NG (1) | 3.45 ± 0.64 (1) | NG (1) | 4.03 ± 0.44 (1) |
| 76°C for 2 min | 0.55 ± 0.64[e] (2) | 4.04 ± 0.48 (2) | 0.62 ± 1.16[e] (2) | 4.25 ± 0.34 (2) |
| 76°C for 3 min | 0.18 ± 0.18[e] (2) | 4.49 ± 0.65 (2) | NG (2) | 4.30 ± 0.38 (2) |
| Room temperature wash for 3 min | 4.47 ± 0.65 (1) | 5.92 ± 0.29 (1) | 5.43 ± 0.07 (1) | 6.65 ± 0.72 (1) |

[a] *E. coli* populations were enumerated on MAC agar medium.
[b] Total aerobic populations were enumerated on TSA agar medium.
[c] Data are reported as the means ± standard deviations for all independent runs. Numbers in parentheses indicate the number of independent runs. Each run consisted of three cantaloupes. NG, no growth was detected under the experimental conditions used.
[d] Cantaloupes were dip inoculated with *E. coli* for 5 min, allowed to air dry in a biosafety cabinet for 2 h, and stored at either room temperature or 4°C for 24 h prior to the washing treatment at the indicated temperature and time.
[e] Although five of six cantaloupes tested showed no growth, 0.1 log CFU/cm$^2$ rind (minimum detection level) was used in place of no growth to determine the mean and standard deviation.

The data presented here showed that surface pasteurization of cantaloupes at 76°C for 2 to 3 min is a potential process for enhancing microbial safety of cantaloupes, as well as increasing the shelf life of this commodity (see "Temperature Penetration Profiles").

The construction of a pathogen research facility at ERRC allowed us to evaluate the commercial-scale surface pasteurization process of cantaloupes inoculated with the human pathogen *Salmonella* Poona RM 2350. This facility consisted of a containment area that housed the commercial-scale equipment and thus eliminated risk of contamination of the laboratory environment. Also, this gave us the opportunity to compare the responses of *E. coli* and *Salmonella* Poona with surface pasteurization treatment by the commercial-scale equipment. Cantaloupes inoculated with *Salmonella* Poona and allowed to dry for 24 h at RT or 4°C were surface pasteurized at 76°C for 3 min (Table 4). There was no significant difference between the controls and the washing treatment at RT for 3 min. This indicated that the washing treatment with water at RT was not able to dislodge or remove *Salmonella* Poona cells attached to the surface of the cantaloupe. The same effect was previously seen with cantaloupes inoculated with *E. coli* (Tables 2 and 3). Resistance to washing with water could be due to the netlike structure of the rind (Fig. 4) and the attachment of *Salmonella* Poona cells to inaccessible sites on the

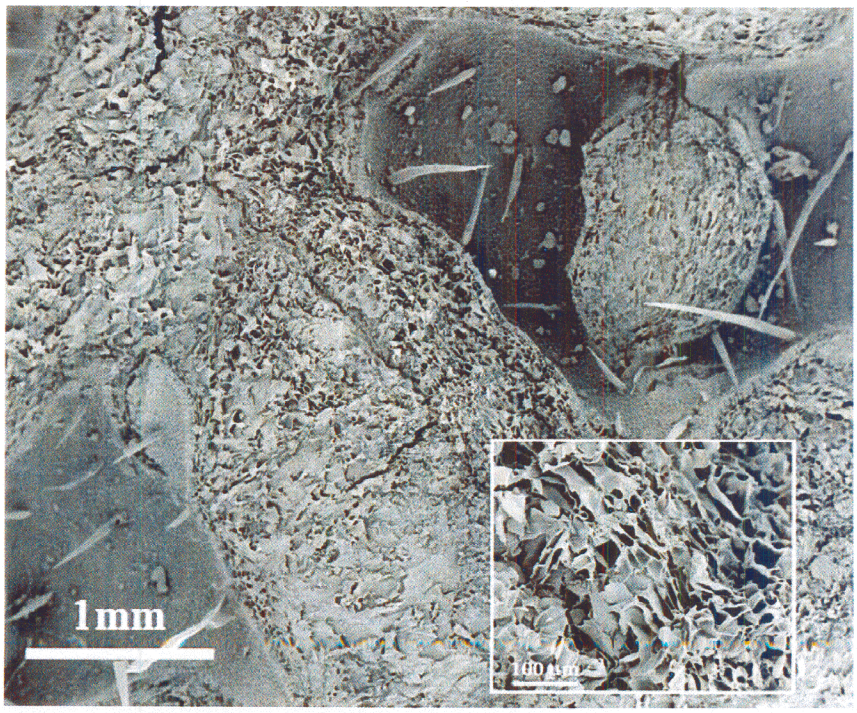


FIGURE 4. *SEM (×25) of a rind of cantaloupe showing an extensive, deeply fissured region of the surface (netting). Inset picture is an enlargement of a section of the netting tissues (×250) showing extensive compartmentation of the tissues.*

TABLE 3. *Comparison of selective[a] and recovery[b] media for enumeration of* E. coli *populations[c] on inoculated cantaloupes following commercial-scale surface pasteurization*

| Treatment[d] | *E. coli* population (log CFU/cm²) | | | |
|---|---|---|---|---|
| | 4°C | | Room temperature | |
| | MAC | TSA with MAC overlay | MAC | TSA with MAC overlay |
| 2-h control | 5.07 ± 0.06 | 5.17 ± 0.07 | 5.07 ± 0.06 | 5.17 ± 0.07 |
| 24-h control | 4.84 ± 0.06 | 4.97 ± 0.03 | 5.14 ± 0.36 | 5.39 ± 0.35 |
| 76°C for 2 min | 0.27 ± 0.24[e] | 0.35 ± 0.35[e] | 0.10 ± 0.00[e] | 0.29 ± 0.27[e] |
| 76°C for 3 min | NG | NG | NG | NG |

[a] *E. coli* populations were selectively isolated on MAC agar medium.
[b] *E. coli* were plated on TSA, plates were incubated for 2 h to allow for recovery of injured cells, then plates were overlaid with MAC and incubated for 24 h.
[c] Data are reported as the means ± standard deviations for three cantaloupes. NG, no growth was detected under the experimental conditions used.
[d] Cantaloupes were dip inoculated with *E. coli* for 5 min, allowed to air dry in a biosafety cabinet for 2 h, and stored at either room temperature or 4°C for 24 h prior to the washing treatment at 76.1°C for either 2 or 3 min.
[e] Although two of three cantaloupes tested showed no growth, 0.1 log CFU/cm² rind (minimum detection level) was used in place of no growth to determine the mean and standard deviation.

rind of the cantaloupe (Figs. 5 and 6), thus avoiding contact with the washing water. Also, *Salmonella* Poona cells seemed to initiate biofilm formation by attachment to the rind of cantaloupe, with fimbriae, following inoculation and drying for 2 h at RT (Fig. 5). Once attached to the rind of the cantaloupe, *Salmonella* Poona cells developed biofilm by growth and excretion of exopolysaccharides following drying for 72 h at RT (Fig. 6). Previously, Annous and Burke *(1)* reported that inaccessibility and biofilm formation were the main factors in failure of the washing treatments to remove or inactivate human pathogens on apples.

Surface pasteurization of cantaloupe at 76°C for 3 min resulted in significantly lower *Salmonella* Poona cell densities compared with controls (Table 4). Although the data indicated that this process did not result in complete inactivation of *Salmonella* Poona cells on the cantaloupe surface, two of the three samples tested showed no growth on selective and recovery media. There was no significant difference between recoveries of *Salmonella* Poona cells on selective and recovery media, indicating that injury was not a factor during surface pasteurization treatments. These results, along with those presented previously for *E. coli* (Tables 2 and 3) indicated that both microorganisms on the surface of cantaloupe were thermally inactivated by this commercial-scale surface pasteurization process. Therefore, along with extending the shelf life of fresh cantaloupe, surface pasteurization of cantaloupe can decrease the risk of foodborne illnesses.

Storage for 24 h at RT and 4°C resulted in significantly higher and lower *Salmonella* Poona cell densities, respectively, compared with the 2-h control (Table 4), suggesting that refrigeration might result in a population reduction of this pathogen. This led to a study of the effect of RT and refrigerated storage for up to 72 h on survival of *Salmonella*

TABLE 4. *Comparison of selective[a] and recovery[b] media for enumeration of* Salmonella *Poona RM 2350 populations[c] on inoculated cantaloupes following commercial-scale surface pasteurization*

| Treatment[d] | *Salmonella* Poona population (log CFU/cm²) | | | |
|---|---|---|---|---|
| | 4°C | | Room temperature | |
| | XLT-4 | TSA with XLT-4 overlay | XLT-4 | TSA with XLT-4 overlay |
| 2-h control | 3.66 ± 0.43 | 5.26 ± 0.40 | 3.66 ± 0.43 | 5.26 ± 0.40 |
| 24 h control | 3.31 ± 0.16 | 5.00 ± 0.32 | 5.54 ± 0.09 | 6.00 ± 0.08 |
| 76°C for 3 min | 0.10 ± 0.00[e] | 0.10 ± 0.00[e] | 0.16 ± 0.08[e] | 0.26 ± 0.22[e] |
| Room temperature wash for 3 min | 4.23 ± 0.32 | 4.91 ± 0.18 | 5.08 ± 0.20 | 5.43 ± 0.19 |

[a] *Salmonella* Poona populations were selectively isolated on XLT-4 agar medium.
[b] *Salmonella* Poona cells were plated on TSA, plates were incubated for 2 h to allow for recovery of injured cells, then plates were overlaid with XLT-4 and incubated for 24 h.
[c] Data are reported as the means ± standard deviations for three cantaloupes.
[d] Cantaloupes were dip inoculated with *Salmonella* Poona for 5 min, allowed to air dry in a biosafety cabinet for 2 h, and stored at either room temperature or 4°C for 24 h prior to the washing treatment at 76°C for either 2 or 3 mn.
[e] Although two of three cantaloupes tested showed no growth, 0.1 log CFU/cm² rind (minimum detection level) was used in place of no growth to determine the mean and standard deviation.

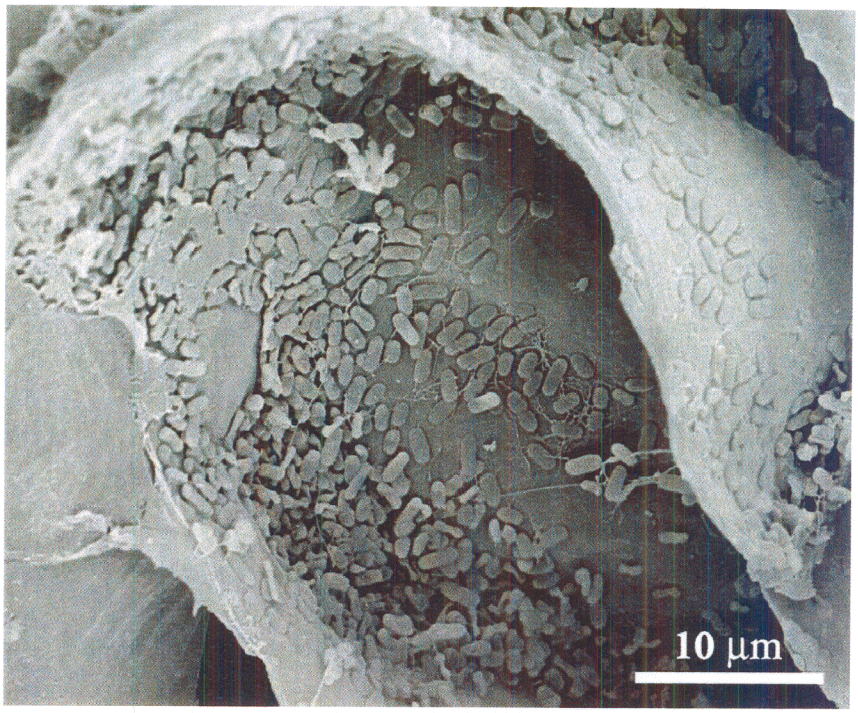


FIGURE 5. *SEM (×2,500) of a rind showing attachment and initiation of biofilm formation by* Salmonella *Poona cells inside the netting of inoculated cantaloupe. Cantaloupes were spot inoculated, allowed to dry for 2 h, dissected, and treated for SEM imaging.*

Poona cells on cantaloupe (Table 5). *Salmonella* Poona cell density on the surface of inoculated cantaloupes following storage at RT for up to 72 h was significantly higher than those stored for 2 h. Inoculated cantaloupes that were stored at RT had significantly higher cell densities than cantaloupes stored at 4°C, which showed up to 1 log reduction. Similar results were reported for *E. coli* O157:H7 inoculated on the rind of cantaloupe *(7)*. Growth of *E. coli* O157:H7 on the rind of a cantaloupe stored at 25°C was supported by nutrients originating from growth medium and peptone *(7)*. Because the culture we used to inoculate cantaloupe was suspended in water, organics that might support growth from the growth medium were not transferred to the surface of the cantaloupe. This suggested that the surface of the cantaloupe was able to support the growth of *Salmonella* Poona at RT. These results are similar to those seen previously (Table 4) and further indicate that refrigeration of cantaloupe can suppress the growth of *Salmonella* Poona. These observations are similar to those reported for *E. coli* O157:H7 inoculated on the rind of cantaloupe and watermelon *(7)*. Cantaloupe should be refrigerated as soon as possible following harvest and during transportation, if possible, to maintain quality and delay spoilage. Here, we showed that refrigeration of cantaloupe could be used to

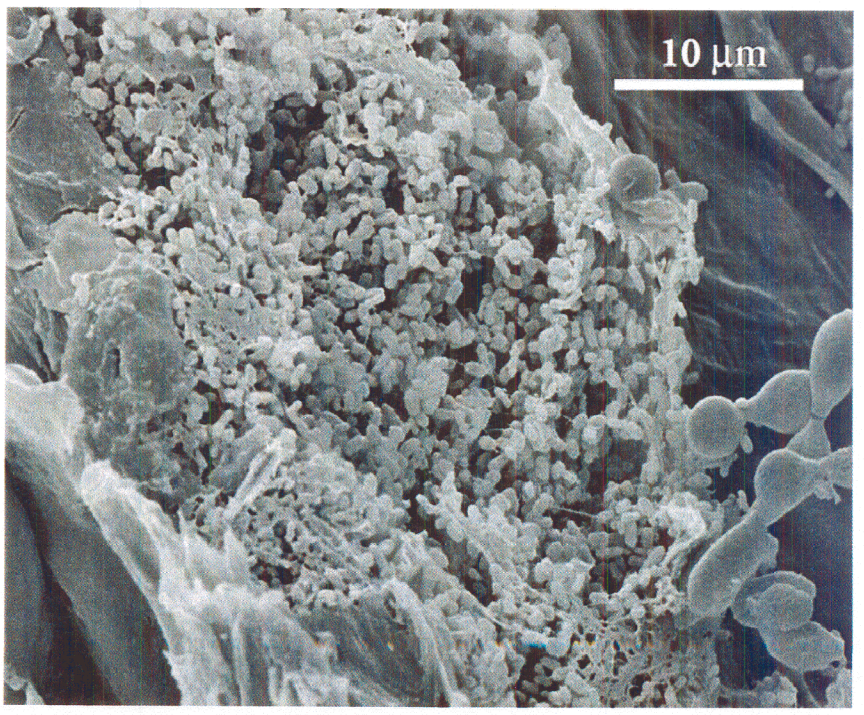


FIGURE 6. *SEM (×2,500) of a rind showing attachment and biofilm formation by* Salmonella *Poona cells inside the netting of inoculated cantaloupe. Cantaloupe was spot inoculated, allowed to dry for 72 h, dissected, and treated for SEM imaging.*

TABLE 5. *Effect of storage temperature on the survival of* Salmonella *Poona RM 2350 cells on inoculated cantaloupes*[a]

| Dry time (h) | RT storage (log CFU/cm$^2$) | 4°C storage (log CFU/cm$^2$) |
|---|---|---|
| 2 | 4.26 ± 0.85 | 4.26 ± 0.85 |
| 24 | 6.72 ± 0.60 | 3.40 ± 0.10 |
| 48 | 6.95 ± 0.24 | 3.08 ± 0.12 |
| 72 | 7.02 ± 0.11 | 3.37 ± 0.39 |

[a] Cantaloupes were dip inoculated and allowed to dry at room temperature or 4°C for up to 72 h. Data are reported as the means ± standard deviations for three cantaloupes. XLT-4 medium was used to enumerate *Salmonella* Poona cell densities. Whole rind was removed with an automatic fruit peeler and was used in enumerating *Salmonella* Poona cell densities.

decrease the microbial safety risk associated with this commodity.

*E. coli* cell densities on cantaloupes stored at RT or 4°C for 24 h showed little change compared with the 2-h controls (Tables 2 and 3), suggesting that *E. coli* cells might be more stable than *Salmonella* Poona cells under the experimental conditions of this study.

**Laboratory-scale surface pasteurization of cantaloupe.** Microbial food safety research that uses human pathogens is usually conducted under laboratory conditions. This research was conducted to compare the efficacy of surface pasteurization of cantaloupe with laboratory-scale equipment to that of commercial-scale equipment. Laboratory-scale washing treatments are usually conducted with the use of small volumes of water or sanitizer solution. This creates a problem in controlling the wash treatment temperatures that was not seen in commercial washing because of the much greater commodity mass/solution volume ratio used. If the commodity is cool or cold, it will bring about a reduction in the treatment temperature through heat transfer from the water. The efficacy of laboratory-scale surface pasteurization of cantaloupes inoculated with *E. coli* and *Salmonella* Poona is shown in Tables 6 and 7, respectively. Storage of inoculated cantaloupes at RT for 24 h resulted in a significant increase in cell densities of contaminants compared with the 2-h controls (Tables 6 and 7). Washing treatments at RT for 3 and 6 min did not result in significant reductions in contaminant populations compared with the 24-h controls (Tables 6 and 7). This was previously seen with the commercial-scale process (Tables 2 and 4) and could be a result of attachment and biofilm formation by these contaminants to inaccessible sites on the surface of the cantaloupe (see "Commercial-Scale Surface Pasteurization of Cantaloupe").

Laboratory-scale surface pasteurization resulted in significant decreases in *E. coli* and *Salmonella* Poona cell densities compared with the controls (Tables 6 and 7), but not as great as determined with the commercial-scale equipment. Treatment for 6 min resulted in significant log reductions compared with 3-min treatments (Tables 6 and 7). The increase in treatment time to 6 min was necessary to improve reduction of the contaminants on cantaloupe. This increase in treatment time was required to compensate for the drop in wash water temperature following addition of a cantaloupe to the wash tank. The addition of one cantaloupe with an average weight of 1,500 g to the wash tank containing 4 liters of water resulted in a rapid temperature drop of up to 5°C. Unlike the commercial-scale equipment, the laboratory-scale equipment is limited by the mass/volume ratio used and thus might result in an underestimation of the process.

Washing cantaloupe at ambient temperature is ineffective in removing microbial contaminants. *Salmonella* Poona cells are able to attach and form biofilms inside the netting of the rind and thus become inaccessible to the washing solution. The work presented here showed that surface pasteurization of cantaloupe is able to thermally inactivate this human pathogen while maintaining the firmness of the mel-

TABLE 6. *Efficacy of laboratory-scale surface pasteurization (76°C) in decontaminating cantaloupes inoculated with* E. coli *ATCC 25922*[a]

| Treatment[c] | Residual population of E. coli ATCC 25922 (log CFU/cm$^2$)[b] | |
|---|---|---|
| | MAC | TSA with MAC overlay |
| 2-h control | 5.28 ± 0.38 | 5.65 ± 0.36 |
| 24-h control | 6.24 ± 0.58 | 6.70 ± 0.39 |
| 76°C for 3 min | 0.83 ± 1.04[d] | 0.81 ± 1.01[d] |
| 76°C for 6 min | NG | 0.51 ± 0.64[d] |
| Room temperature wash for 3 min | 6.34 ± 0.23 | 6.69 ± 0.48 |
| Room temperature wash for 6 min | 6.10 ± 0.20 | 6.29 ± 0.26 |

[a] *E. coli* populations were enumerated on selective medium (MAC) and recovery medium (TSA with MAC overlay).

[b] Data are reported as the means ± standard deviations of two independent runs. Each run consisted of three cantaloupes. NG, no growth was detected under the experimental conditions used.

[c] Cantaloupes were dip inoculated with *E. coli* for 5 min, allowed to air dry in a biosafety cabinet for 2 h, and stored at room temperature for 24 h prior to the washing treatment at 76°C for either 3 or 6 min.

[d] Although four of six cantaloupes tested showed no growth, 0.1 log CFU/cm$^2$ rind (minimum detection level) was used in place of no growth to determine the mean and standard deviation.

TABLE 7. *Efficacy of laboratory-scale surface pasteurization (76°C) in decontaminating cantaloupes inoculated with* Salmonella *Poona RM 2350*[a]

| Treatment[c] | Residual population of *Salmonella* Poona RM 2350 (log CFU/cm$^2$)[b] | |
|---|---|---|
| | XLT-4 | TSA with XLT-4 overlay |
| 2-h control | 4.88 ± 0.29 | 5.62 ± 0.37 |
| 24-h control | 5.92 ± 0.49 | 6.34 ± 0.18 |
| 76°C for 3 min | 1.48 ± 0.99[d] | 1.71 ± 1.18[d] |
| 76°C for 6 min | 0.33 ± 0.33[e] | 0.46 ± 0.50[e] |
| Room temperature wash for 3 min | 5.47 ± 0.14 | 6.04 ± 0.16 |
| Room temperature wash for 6 min | 5.61 ± 0.47 | 5.85 ± 0.56 |

[a] Poona populations were enumerated on selective medium (XLT-4) and recovery medium (TSA with XLT-4 overlay).
[b] Data are reported as the means ± standard deviations of two independent runs. Each run consisted of three cantaloupes.
[c] Cantaloupes were dip inoculated with *Salmonella* Poona for 5 min, allowed to air dry in a biosafety cabinet for 2 h, and stored at room temperature for 24 h prior to the washing treatment at 76°C for either 3 or 6 min.
[d] Although two of six cantaloupes tested showed no growth, 0.1 log CFU/cm$^2$ rind (minimum detection level) was used in place of no growth to determine the mean and standard deviation.
[e] This treatment consisted of one run of three cantaloupes. Although two of three cantaloupes tested showed no growth, 0.1 log CFU/cm$^2$ rind (minimum detection level) was used in place of no growth to determine the mean and standard deviation.

on. The process also helped increase the shelf life of cantaloupe by reducing the spoilage microorganism populations on the rind surface. Furthermore, the work presented here indicated that the rind of the cantaloupe can support the growth of microorganisms, including *Salmonella* Poona. Therefore, cantaloupe should be refrigerated as soon as possible following harvest to suppress the growth of microorganisms on the rind and thereby decrease the risk of foodborne illness and improve shelf life. Currently, we are optimizing this commercial-scale treatment to enhance microbiological safety while maintaining the freshness of cantaloupe. Also, we are developing new commercial-scale washing equipment compatible with this process and modifying existing equipment to increase washing efficiency.

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